Vol. XXIII. *August, 1912.* *No. 3*

BIOLOGICAL BULLETIN

THE INTERSTITIAL CELLS AND THE SUPPOSED INTERNAL SECRETION OF THE CHICKEN TESTIS.[1]

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The problem presented in this paper was suggested to me by Dr. Pearl in connection with some work of his on the time when the secondary sexual characters first appear in the domestic chicken. The domestic chicken has a long list of secondary sexual characters. Will the cytology of the reproductive organs throw any light on the cause of the development of these secondary characters? The observations described in this paper deal with the cytology of the testis, with especial reference to the problem of secondary sexual characters.

The problem of the cause of the secondary sexual characters has been approached in many ways, but the favorite one is the internal secretion theory, that is, that the secondary sexual characters are dependent on a secretion formed by the cells of the primary reproductive organs. Some phases of this theory consider the germ cells themselves as the source of this secretion, but oftener the so-called interstitial cells are regarded as the secreting agents. We find frequent references to these interstitial cells in the literature, but there has been great confusion between the terms "interstitial tissue" which might be any connective tissue lying between the seminal tubules, and the term "interstitial cells," which by some workers is used for gland cells among the connective tissue between the tubules. Workers disagree as to whether these gland cells are epithelial or mesenchymous in origin. Another term frequently used is "interstitial gland" which seems to be used for any large accumulation of the

[1] Papers from the Biological Laboratory of the Maine Experiment Station, No. 38.

gland cells in the interstitial tissue. Poll, in his studies of hybrid birds, speaks of the increased development of the interstitial gland composed of cells of epithelial nature in contradistinction to the connective tissue. Ceni, in his account of experiments with feeding ducks on caffeine and removing the cerebrum, speaks of an increase of the amount of interstitial tissue at the expense of the cells of the seminal tubules and mentions groups of epithelial cells called interstitial glands. On the other hand, Loisel distinctly states that he finds no evidence of activity in the cells of the interstitial tissue of birds at any time of year, but finds a fatty secretion formed by the Sertoli cells. Mazzetti finds no more evidence of secretory granules in the cells of the interstitial tissue than in the cells of the seminal tubules. He claims that interstitial cells are derived from connective tissue cells and gives an interesting list of variations in the number of interstitial cells in different animals and in the same animal at different times of year. Kirkbride states that the number of interstitial cells in the testes of newborn infants is very variable. Gudernatsch describes a case of hermaphroditism in man where the interstitial tissue of the testicular portion of the ovotestis was enormously developed, and yet the secondary sexual characters were such that the individual was usually regarded as a female.

So the question of the origin, nature and function of the interstitial cells has been by no means settled. Now if it is a general law that the interstitial cells and secondary sexual characters are causally interrelated, one would certainly expect to find abundant interstitial cells in a bird with such marked secondary sexual characters as the domestic chicken.

Methods.

Just-hatched chicks were used first, as there are some indications that there may be a differentiation in respect to certain secondary sexual characters at that early age. The chicks were all Barred Plymouth Rocks from the Maine Experiment Station stock, not a day old. The gonads are easily cut off from the surface of the kidney with scissors. They were fixed in Gilson's, Flemming's and Hermann's solutions, these two osmic agents being used especially because the internal secretion of the testis has usually been described as appearing in the form of fat or oil drops in the cells.

For comparison with these, I have studied the testes of fourteen older birds, ranging from 5 to 12 months in age. These were all but three pure Barred Plymouth Rocks, one was a White Leghorn, and two were cross-breds. They were killed at different times of year, October, November, January, March and April. In birds over six months of age, dividing germ cells were found, all stages from spermatogonia to spermatozoa. The testes from six of these birds had been put up previously for other purposes for which the best cytological methods were not necessary, but the general observations on these accord so well with those on the very best fixed material, that I do not hesitate to state that my observations cover fourteen birds. The other eight testes were cut in small pieces and fixed in Gilson, Flemming and Hermann. With two birds, every possible precaution to secure absolutely normal fixation was taken: the testis was removed from the living bird and put directly into Flemming, heated to the temperature of the bird's body, and the testis was then cut into thin slices with a razor, as it was held in the hot Flemming.

The ordinary method of clearing in xylol for embedding and mounting was not used with all of the material, as xylol dissolves fat. From Mallory and Wright's "Pathological Technique," I found that chloroform and clove oil do not dissolve fat fixed in osmic acid, so by clearing the Flemming material in chloroform or clove oil, and mounting the sections in chloroform balsam, I got preparations showing fat.

Observations.

In sections of the just-hatched testis, one can see without great magnification that about half the substance is interstitial connective tissue (Fig. 1). The seminal tubules are small and far apart. Careful detailed study shows that the cells of the seminal tubules have comparatively large round nuclei, with a linin network and scattered chromatin granules; in fact, they are typical resting germ cells. There were no signs of any dividing cells, or of any spermatocytes or spermatozoa. These cells are evidently quiescent early spermatogonia.

The abundant interstitial substance shows no marked differ-

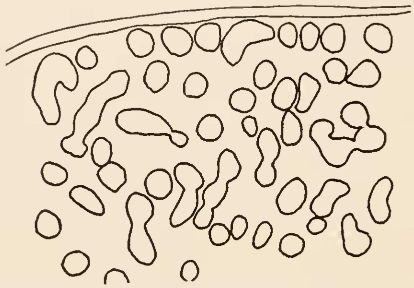

FIG. 1. Section through testis of just-hatched chick, showing seminal tubules in outline, interstitial spaces, and testis sheath. × 100.

entiation. It looks like embryonic connective tissue (Fig. 2). The fibers run in all directions. The nuclei are mostly round, but some of them are elliptical or irregular in shape. However,

FIG. 2. Portion of Fig. 1, showing nuclei and fat (*F*) in interstitial tissue. *C* = blood corpuscles. × 1,000,

the nuclei in the connective tissue sheath of the testis are all typical elliptical connective tissue nuclei. Why this difference? One cannot help noticing that the texture of the interstitial substance where the nuclei are mostly round is loose and reticular, while the fibers of the sheath lie parallel and close together, and look as though they were stretched tight around the testis. This suggests that the difference in the shape of the nuclei may be due to difference in pressure relations.

Further, none of these cells of the interstitial substance show any signs of glandular activity. The so-called "interstitial cells" have been described in a recent paper by Mazzetti as grouped in complexes in the intercanalicular spaces, as polyhedral or ovoid with abundant protoplasm, large round nucleus, an evident nucleolus, protoplasm in two zones, a clear peripheral and an intensely-coloring central, also as containing secretory granules, either crystalline or fatty in nature. There are no such cells in the interstitial substance of the testis of just-hatched chicks, and the connective tissue cells which make up this substance show no sign of glandular activity. In sections of material cleared in clove oil so as not to dissolve fat, great masses of fat stained black by osmic appear in the interstitial tissue, but they are not grouped about any especial cell, and have no appearance of being secreted by any of these cells (Fig. 2).

This being the condition in the quiescent testes of the just-hatched chick, we may next compare with it the testes of birds in which active spermatogenesis is going on.

In the older birds, there is a striking individual variation in the size of the seminal tubules. This variation has no relation to breed, age, time of year, or apparent stages of the germ cells, but occurs in birds of the same breed, and age, killed on the same day, with the germ cells in both showing all stages of spermatogenesis. However, there is one constant relation, the relation of the size of the seminal tubules to the amount of interstitial tissue. Wherever the seminal tubules are small the interstitial spaces are wide, and where the seminal tubes are large, the interstitial spaces are narrow. Figures 3 and 4 bring out this point. It is not only the width of the spaces between any two tubules that varies but also the size of the triangular spaces where any three or four tubules come into juxtaposition. This relation can be proved by accurate measurements.

For these measurements, I used slides from the testes of four Barred Plymouth Rock cockerels of about the same age, killed on the same day, all in active spermatogenesis. The measuring was done with an eyepiece micrometer. One hundred seminal

FIG. 3. Section of testis of ♂ 666, showing small tubules and wide spaces. × 100. *ABC* = one triangle in which the cells were counted, such as Fig. 5 represents.

tubules were measured in each of the four birds, and 100 spaces between tubules. There seems to be no accurate way to measure the triangular spaces.

FIG. 4. Section of testis of ♂ 147, showing large tubules and narrow spaces. × 100.

A glance at Table I. will show the relation between the two sets of means: taking the birds in the order nos. 666, 1271, 2323, 147, the tube size increases, and the space width decreases.

Table I.

Absolute Measurements and Cell Counts of Testicular Elements. Mean Values.

Bird No.	Tube Width.	Space Width.	No. Cells in Triangle.	No. of Long Cells.	No. of Round Cells.	No. of Light Cells.	No. of Dark Cells.	No. of Dark Long Cells.	No. of Dark Round Cells.
666	45.43	3.46	67.68	33.16	34.52	59.00	8.68	3.28	5.40
1271	50.82	3.015	60.76	27.32	33.44	58.92	1.84	.24	1.60
2323	82.99	1.455	23.16	14.92	8.24	20.60	2.56	.80	1.76
147	93.14	1.385	50.96	33.68	17.28	50.08	.88	.48	.40

Table II.

Relative Proportions of Different Cell Elements.

Bird No.	Per Cent. Long Cells.	Per Cent. Round Cells.	Per Cent. Light Cells.	Per Cent. Dark Cells.	Per Cent. Dark Long Cells	Per Cent. Dark Round Cells.
666	48.98	51.02	87.17	12.83	37.79	62.21
1271	44.96	55.04	96.80	4.20	13.27	86.73
2323	64.42	35.58	88.94	11.06	31.25	68.75
147	66.09	33.91	98.27	1.73	54.54	45.46

On examining the interstitial spaces in these four testes with greater magnification, there appears to be what one would naturally expect, a greater number of cells in the larger triangles and wider spaces. An accurate count of the cells shows this to be the fact. The cells in twenty-five triangles of each of the same four testes were counted. In order to count over comparable areas the cells were counted from the points of the triangle along the spaces between each two tubules to about half way to the next triangle (Fig. 3, *A*, *B*, *C*). The sections in the four testes were of course cut the same thickness. A study of Table I. shows that the number of cells varies with the width of spaces and inversely as the size of the tubules, with one exception. That is, arranged according to mean number of cells in spaces, the birds stand in the order 666, 1271, 147, 2323, as contrasted with 666, 1271, 2323, 147, the order for tubule size. The exception is in the reversal of 147 and 2323. The explanation of this lies in the fact that the measurements are of the spaces between two tubules and the count of cells includes the triangular space between three tubules. There is very little difference in the space width of 147 and 2323, but the triangles of 2323 are decidedly smaller.

This difference in tubule size is so striking and seems so strange in birds so nearly alike in other ways, that I tried to find any possible other explanation than that of individual variation. It occurred to me that possibly the size of tubules might vary in different parts of the testes, and as the pieces of testes first sectioned were cut out of the organ without any reference to position the probability is that they were from different parts in the four different testes. However, in two birds killed, I carefully fixed pieces from the periphery and from the center of the organ, as these two regions would represent any difference in pressure conditions or in growth. The sections of these pieces

FIG. 5. Triangular space of interstitial tissue in ♂ 666, showing many nuclei of different shapes and staining capacity, also many fat globules arranged in rows. × 1,000.

show that the tubule size is the same throughout. Though the testis from which Fig. 3 is drawn has many fully formed spermatozoa, it is just possible that it has not reached the climax of growth, and would eventually have contained tubules as large as those in Fig. 4.

In the adult testis, the nuclei vary in shape exactly as in the just-hatched chicks, and there are here also no cells differentiated from the connective tissue fibers. In the adult there are more elongated nuclei and fewer round ones. In the testis, where the tubules are large, and crowded close together, so that there is not much interstitial tissue between them, the nuclei are mostly elliptical, with some round ones in the triangle (Fig. 6). Where the tubules are small and far apart, leaving much interstitial tissue there are more round nuclei, and they lie not only in the

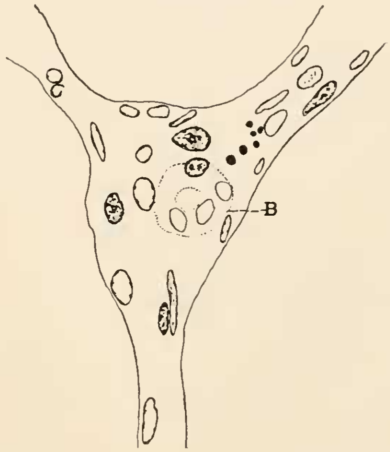

FIG. 6. Same as Fig. 5, but from ♂ 2323, showing few nuclei and few fat globules. *B* = blood vessel. × 1,000.

triangles (Fig. 5). In counting the nuclei as mentioned above, I differentiated between long and round ones. Table I. shows the mean number of each kind, and Table II. gives the percentage of each shape to the total number of nuclei. In ♂s 666 and 1271, with the small tubules, 48 per cent. and 44 per cent. nuclei are long, but in ♂s 147 and 2323 where the tubules are large, 66 per cent. and 64 per cent. are long. This relation must mean a difference in mechanical conditions of pressure.

If the difference in the cells of the interstitial spaces is in the shape of the nuclei, and the shape of the nuclei is merely due to mechanical causes, can we call any of these cells interstitial cells with the accepted meaning of the word? We could form a complete series of nuclei ranging gradually from the typical elliptical connective tissue nuclei to the round undifferentiated nuclei (Figs. 2, 5, 6). From the figures and statements in Mazzetti's paper, it appears that he has made the shape of the nucleus the important factor in deciding which cells are interstitial cells. But his results, in entire agreement with those of the present paper, show a complete series of gradations from his so-called interstitial cells with round nuclei to typical connective tissue cells. Also he finds a variation in the proportion of interstitial cells in animals of widely different species. Here in the chicken there is as much variation in this regard in birds of the same breed, if we take the shape of the nuclei as the basis for deciding which are interstitial cells, as Mazzetti describes for different genera or orders.

The only other difference evident in the cells of the interstitial tissue is the staining capacity of the nuclei. This difference is

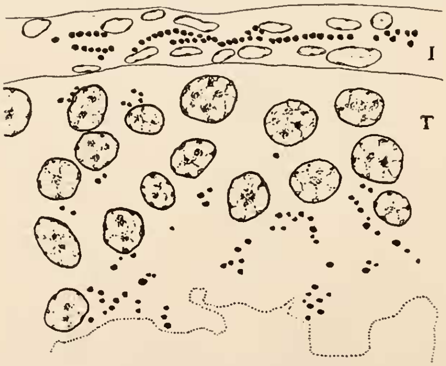

Fig. 7. Section of testis in ♂ 666, showing part of one seminal tubule (*T*) and the adjacent interstitial tissue (*I*). The fat is indicated by black dots. × 1,000.

not noticeable in just-hatched chicks, but very apparent in the older birds. Some stain very dark, and some remain almost unstained. Table I. gives the mean number and Table II. the percentage of dark and light cells. In ♂s 666, and 1271, there are 12 per cent. and 4 per cent. dark cells, and in ♂s 2323 and 147, there are 11 per cent. and 1 per cent. That would be a great variation in the per cent. of interstitial cells, if a dark staining nucleus could be regarded as a distinctive character of an interstitial cell. But it cannot. I made a differential count of the long and round nuclei among the dark staining ones, and found

37 per cent., 13 per cent., 31 per cent. and 54 per cent. of the dark-staining nuclei which have the long shape of typical connective tissue nuclei. It seems then that staining capacity of nuclei is not a satisfactory method of distinguishing interstitial cells.

There is one more point in the frequent descriptions of interstitial cells to be considered. Is there any evidence that the cells of the interstitial tissue are secreting fat? We have already found fat present in testes of just-hatched chicks, but decided that it probably was not a secretion. Fat is also present in

FIG. 8. Same as Fig. 7, but from ♂ 2323. *S* = spermatozoa. × 1,000.

the older testes, and is represented by the black dots in the drawings. Comparison of Figs. 5 and 7 with Figs. 6 and 8 will show that the fat is inside of the tubules as well as in the interstitial tissue. The fat globules in the interstitial tissue are not arranged like secretory granules in the cytoplasm of cells. They are mostly in long rows as though packed in spaces between connective tissue fibers. Figs. 5 and 7 show nearly as much fat near typical connective tissue nuclei as near the round or dark-stained nuclei of the "interstitial cells." A comparison of Figs. 7 and 8 shows much fat in Fig. 7 where there are only connective tissue nuclei in the interstitial space, and no fat in Fig. 8, where there is a small group of round dark-staining nuclei. Fig. 9 shows a part of a large mass of interstitial tissue where the nuclei are mostly round. This is evidently a so-called interstitial gland.

If interstitial cells or cells of interstitial connective tissue are secreting the fat found in the testis we certainly would expect to find it abundant in such an "interstitial gland," or if we prefer not to use that term, in so large a mass of interstitial tissue. However, there is not so much fat as in some of the smaller triangles and narrow spaces. This study shows *no evidence that the fat in the active testis is formed by the interstitial cells.*

FIG. 9. Part of an "interstitial gland" in ♂ 666, showing many round nuclei, but comparatively little fat. × 1,000.

It seems likely that this fat is being brought to the testis by the general metabolic processes possibly in connection with sexual activity, just as fat is deposited in the yolk of eggs in the hen. This would fit in with the former suggestion that possibly the testis in ♂ 666 is not as far developed as 147 and 2323. The tubules in 666 are smaller and the quantity of fat is greater. It may be in the process of active growth, while 2323 has reached the climax of growth of tubules, and used up most of the fat for this growth.

SUMMARY.

In conclusion, then, I find no cells in the interstitial tissue in the young or old chicken testis with the cell bodies differentiated from the connective tissue fibers. No evidence has been found that differences in shape of the nuclei are indicative of functional differences in the various cells of the interstitial tissue. On the contrary, it appears that these differences in shape depend on mechanical pressure conditions. The difference in the staining capacity of the nuclei is not a basis for cell classification, for the nuclei which take a deep stain include those of all shapes. The fat in the testes is probably not formed by the interstitial tissue at all, but is brought there by the circulation and deposited. So we are brought to the conclusion that there are no "interstitial cells" in the testis of the domestic chicken in the sense that this term has been previously used. Furthermore no evidence has been found in this study which would indicate that an internal secretion of any kind is formed by any cells of the interstitial tissue. This result would appear to derive interest and significance in consideration of the fact that in few animals is there so extensive a development of secondary sexual characters as in the male of the domestic fowl.

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